ERYTHROCYTE MEASUREMENTS IN FISHES, AMPHIBIA, AND REPTILES [1]

F. A. HARTMAN AND M. A. LESSLER

Department of Physiology, The Ohio State University, Columbus 10, Ohio

Gulliver (1875) made an extensive study of the size of the red cells in vertebrates, extending over a period of years. Cullen (1903) gives values for the skate and dogfish, while Cleland and Johnston (1912) reported a considerable series, especially birds. Although Wintrobe (1961) tabulated the results of many investigators on red cell size, there have been relatively few reports of new red cell measurements and many species remain unreported.

The size and shape of red cells gives an indication of the surface available for the exchange of gases in respiratory functions. Study of erythrocytes in many different species provides an interesting comparison of red cell size in relation to activities and habits. While collecting specimens for adrenal studies, we were frequently able to make fresh blood smears from the animals. These were air-dried and later stained and studied. The material was obtained in Panamá and the United States.

Materials and Methods

Blood smears were made immediately after the death of the animal. These were air-dried promptly and stored until prepared for study in the laboratory. Staining was carried out under controlled conditions. Five drops of standard Wright's stain were allowed to remain on the slide for one minute before addition of a pH 6.5 buffer. The slide was allowed to stand for five minutes at room temperature and then washed with distilled water for 30 seconds and allowed to dry.

Ten cells on each slide, selected for excellence of staining and internal cytology, were measured by means of a calibrated eyepiece used in conjunction with an oil-immersion objective. In this way ten measurements of maximum cell width and length, as well as nuclear width and length, were made. The ratios of cytosome length to width and nucleus length to width have been calculated. This is a measure of cell and nuclear deviation from the spherical shape.

Erythrocytes of eight species among seven families of fishes, five species among five families of Amphibia, and thirty-three species among seven families of reptiles were measured (Table I). When measurements were made of only one individual of a species, the standard error of values for the different cells is shown. When

[1] Fishes were collected at the Marine Biological Laboratory, Woods Hole, Massachusetts. Other material was obtained in Ohio; in Florida with the Archbold Biological Station as a base; and in Panamá under the auspices of the Smithsonian Institution. We are indebted to Richard Archbold for his assistance, to Dr. Carl Johnson for the use of the Juan Mina Field Station of the Gorgas Memorial Institute and to Alois Hartmann for facilities near Volcan in West Panamá.

TABLE I

Erythrocyte measurements in fishes

Family and species	Cytosome		Ratio L/W	Nucleus		Ratio L/W
	Length μ	Width μ		Length μ	Width μ	
Rajidae						
Raja erinacea	20.3 ±1.5	12.9±1	1.57	8.4± .8	6.1± .3	1.37
Dasyatidae						
Dasyatis centrura (4)	19.7 ± .38	13.8±0.24	1.42	8.1±0.24	6.9±0.24	1.17
Anguillidae						
Anguilla rostrata	11.5 ±0.3	7.6±0.3	1.51	3.8±0.2	3.2±0.5	1.19
Leptocephalus conger	10.9 +1.5	7.7±1	1.43	4.0±0.5	3.3±0.5	1.21
Labridae						
Tautogo onitis (3)	10.5 ±0.35	7.3±0.18	1.44	4.1±0.15	3.0±0.15	1.37
Triglidae						
Prionotus carolinus (4)	11.15±0.15	7.3±0.32	1.53	4.8+0.28	3.0±0.15	1.37
Batrachoididae						
Opsanus tau	14.9 ±2	12.9±2	1.15	5.8+0.8	5.2±1	1.11
Lophiidae						
Lophius piscatorius	11.6 ±2	10.3±1	1.13	5.1+0.5	4.6±0.5	1.11
	13.6 +1.5	11.2±1	1.19	5.3+0.8	4.7±1	1.13

three or more individual specimens of a species were measured, the standard error is based on the averages of the individuals involved.

RESULTS

Among the fishes studied, the elasmobranch erythrocytes are nearly twice the size of the erythrocytes in the teleosts, and their width is a little more than half their length. The cytosomes of *Opsanus* and *Lophius* are almost as wide as long (ratio 1.15 to 1.19). In all of the fishes the ratio of length to width of the nucleus

TABLE II

Erythrocyte measurements in Amphibia

Family and species	Cytosome		Ratio L/W	Nucleus		Ratio L/W
	Length μ	Width μ		Length μ	Width μ	
Amphiumidae						
Amphiuma tridactylum	65.3±1.27	36.6±0.39	1.78	26.4±0.76	16.8±0.45	1.57
Proteidae						
Necturus maculosus	56.4±0.77	38.1±0.76	1.48	30.2±0.85	17.4±0.32	1.74
Bufonidae						
Bufo marinus	18.5±1.04	12.5±0.30	1.48	7.4±0.56	5.2±0.46	1.42
	19.2±0.91	13.4+0.53	1.43	7.6±0.38	5.6±0.41	1.36
Hylidae						
Hyla gratiosa	20.3±0.22	13.4±0.27	1.52	7.4±0.17	4.5±0.15	1.64
Ranidae						
Rana catesbiana	27.9±0.45	15.4±0.40	1.81	10.3±0.31	5.6±0.27	1.84

shows little range (1.11 to 1.37), the width being somewhat less than the length.

The tailed Amphibia possess by far the largest erythrocytes observed in our study (56 μ to 65 μ by 37 μ to 38 μ) while the erythrocytes of the anurans is one-half this size or smaller (19 μ to 28 μ by 13 μ to 15 μ) (Table II). The ratio of length to width in the cytosomes is 1.5 or larger. The ratio of length to width in the nuclei is of similar range.

Among the reptiles, in the one species of crocodilids studied, the cytosome and nuclear measurements are similar to those in elasmobranchs except that the ratios of length to width in the cytosomes and nuclei are greater (Table III). In the lizards there are considerable differences. Reptilian red cell lengths range from

TABLE III

Erythrocyte measurements in a crocodilian and in lizards

Family and species	Cytosome		Ratio L/W	Nucleus		Ratio L/W
	Length μ	Width μ		Length μ	Width μ	
Crocodilidae						
Cayman sclerops (3)	20.6±0.59	10.9±0.46	1.88	7.1±0.19	4.1±0.57	1.74
Iguanidae						
Anolis carolinensis	14.8±0.42	10.3±0.39	1.44	6.0±0.06	3.8±0.18	1.59
Anolis polylepis (3)	18.4±0.47	10.1±0.87	1.84	7.6±0.29	4.8±0.27	1.58
Anolis pachypus	15.6±0.83	8.3±0.44	1.88	6.1±0.75	3.0±0.17	2.03
Anolis biporcatus (3)	18.5±0.37	12.0±0.64	1.54	8.0±0.18	5.2±0.10	1.54
Sceloporus malachiticus (6)	16.9±0.44	9.8±0.27	1.72	6.6±0.25	3.5±0.23	1.89
Basiliscus americanus	19.3±1.04	11.6±0.57	1.60	7.6±0.42	6.4±0.40	1.19
	18.6±0.31	12.1±0.47	1.54	6.5±0.15	5.5±0.12	1.18
Iguana iguana	15.4±0.28	7.5±0.37	2.06	5.6±0.24	4.5±0.35	1.34
Teiidae						
Anadia ocellata	16.3±1.18	9.0±0.76	1.81	5.7±0.35	3.2±0.27	1.78
Cnemidophorus sexlineatus	15.8±0.32	9.3±0.20	1.70	6.4±0.15	3.0±0.12	2.11
Ameiva praesignis (3)	15.0±0.16	8.6+0.46	1.75	5.9±0.23	3.5±0.20	1.66
Scincidae						
Eumeces fasciatus	15.9±0.16	8.5±0.16	1.87	5.1±0.16	3.1±0.10	1.67

15 μ to 19 μ with widths from 7.5 μ to 12 μ. Their length to width ratios vary from 1.44 to 2.06. Likewise there is a great range between nuclear measurements (5.1 μ to 8 μ) and their length to width ratios are from 1.19 to 2.11.

In general, erythrocytes of snakes are large (15.5 μ to 23 μ); some are even larger than those in elasmobranchs. The cytosome length to width ratios range from 1.2 to 1.86 and the nuclei length to width ratios from 1.35 to 2.5. Measurements in three species of *Bothrops* indicated that their erythrocytes are the largest of all reptiles studied (Table IV).

In order to permit free movement of erythrocytes we can assume that the diameter of the smallest capillaries must be no less than the smaller diameter of the erythrocyte. With this assumption it is interesting to compare the minimal capillary diameters of different species and groups of vertebrates. The capillaries of fishes range from 7.3 μ to 13.8 μ in diameter. The largest capillaries are found in the elasmobranchs and the smallest among the bony fishes. The capillaries of Amphibia would range from 12.5 μ to 13.4 μ, while those of reptiles would range from 7.5 μ to 13.8 μ. The smallest are found in the lizards.

Discussion

Cytosome size

Since the erythrocyte is the most important carrier of oxygen and carbon dioxide, its surface area to size ratio is a determining factor in the exchange of these gases with the tissues. Thus, a small corpuscle offers the possibility of a greater rate of exchange than a larger one. Likewise an elliptical body is more efficient than a spherical one of the same volume. Based strictly on geometrical considerations, the red cells of the bony fishes would be the most efficient and those of the tailed amphibians the least efficient. Compared with warm-blooded animals the erythrocytes of these poikilothermic animals would be much less efficient.

Table IV

Erythrocyte measurements in snakes and turtles

Family and species	Cytosome		Ratio L/W	Nucleus		Ratio L/W
	Length μ	Width μ		Length μ	Width μ	
Colubridae						
Spilotes pullatus	17.7±0.24	10.3±0.18	1.72	7.1±0.19	3.7±0.20	1.92
Dryadophis boddaerti	16.3±0.55	11.0±0.92	1.48	7.0±0.63	4.5±0.83	1.56
Masticophis flagellum (3)	16.5±0.23	12.6±0.49	1.31	6.8±0.12	4.2±0.11	1.62
Clelia clelia (3)	20.2±0.45	11.0±0.22	1.82	7.3±0.40	3.7±0.11	1.97
Erythrolamprus bizonus	19.8±0.34	13.0±0.27	1.52	8.0±0.27	3.7±0.09	2.16
Leimadophis epinephalus	16.9±0.94	11.7±0.52	1.44	7.7±0.45	3.6±0.33	2.14
Leimadophis taeniurus	19.2±0.46	12.3±0.13	1.56	7.8±0.34	3.1±0.13	2.52
Thalerophis occidentalis	15.5±0.20	11.7±0.28	1.32	6.0±0.59	4.2±0.32	1.43
Dendrophidion paucicarinatus	17.1±0.04	12.5±0.76	1.36	6.6±0.70	3.8±0.50	1.73
Thamnophis sauritus	16.4±0.23	13.4±0.27	1.22	7.4±0.17	4.5±0.15	1.64
Natrix sipendon	18.2±0.84	13.8±1.28	1.41	6.8±0.74	4.5±0.74	1.51
	18.4±0.35	12.3±0.25	1.49	7.4±0.24	4.3±0.09	1.72
Natrix taxispilota	18.5±0.28	11.7±0.26	1.58	5.8±0.11	4.3±0.12	1.35
Lampropeltis doliata	18.1±1.02	11.8±0.96	1.53	7.1±0.84	4.4±0.48	1.61
Elaphe guttata	18.9±0.28	11.8±0.16	1.59	6.2±0.14	3.6±0.13	1.72
Coluber constrictor (4)	15.3±0.11	12.1±0.34	1.26	6.7±0.28	3.8±0.08	1.76
Opheodrys aestivus	16.3±0.29	12.5±0.19	1.30	6.9±0.15	4.6±0.42	1.50
Viperidae						
Bothrops lateralis	22.2±0.39	11.9±0.17	1.86	5.9±0.12	4.0±0.07	1.47
Bothrops nummifer	23.4±1.01	13.6±0.78	1.72	7.5±0.25	4.8±0.50	1.56
Bothrops atrox	22.1±0.96	12.6±0.46	1.75	7.7±0.55	4.9±0.24	1.57
Testudinidae						
Gopherus polyphemus (3)	19.1±0.61	11.2±0.22	1.71	6.7±0.55	4.0±0.12	1.69
Pseudemys ornata	18.6±0.01	11.6±0.76	1.60	5.5±0.35	5.2±0.66	1.06

In mammals the non-nucleated disc-shaped red cells have diameters from 5 μ and 6 μ (horse, cow, pig, mouse and rat) to about 7.5 μ (chimpanzee, woodchuck and llama) (Wintrobe, 1961). In birds the nucleated elliptical erythrocytes have size ranges from 10.7 μ by 6.1 μ to 15.8 μ by 10.2 μ (Hartman and Lessler, 1963).

The red cell count in poikilotherms is low. Counts of 70,000 to 390,000 per $mm.^3$ in elasmobranchs; 780,000 to 1,490,000 in bony fishes; 20,000 to 440,000 in Amphibia; 560,000 to 1,050,000 in reptiles have been reported. In homiotherms, the red cell count in birds ranges between 1,930,000 and 3,690,000 per $mm.^3$ and in mammals it is much more variable, ranging from 6,300,000 per $mm.^3$ in the chimpanzee to 18,000,000 per $mm.^3$ in the llama (Wintrobe, 1961).

Cleland and Johnston (1912) measured the erythrocytes in Australian vertebrates. By far the largest erythrocytes they reported were found in the lung fish, *Ceratodus* (39 μ by 25 μ). The elasmobranch erythrocyte sizes they reported are similar to our measurements, as are the teleost erythrocytes, except those of *Terapion* which are quite small (6 μ to 7 μ by 6 μ to 7 μ). The erythrocytes of the lizards Cleland and Johnston measured showed a somewhat greater range in size than our measurements, but their other reptile erythrocyte measurements are similar to those of our specimens.

Nuclei

In all fish erythrocytes the nuclei are shorter in proportion to their width than are the cytosomes. In the amphibian erythrocyte nuclei there is usually little difference in these ratios. This is also true in one turtle and in *Cayman*. In lizards, six out of ten species possessed relatively rounder nuclei than cytosomes, while in snakes all but four species had nuclei longer than the cytosomes. Nuclei were relatively longer than the cytosomes in all but six species of birds, being shorter in four and doubtful in two (Hartman and Lessler, 1963). Longer nuclei could offer a somewhat greater surface for exchange with the cytoplasm. This may be a significant factor in nuclear-cytoplasmic exchange. Actually the role of the nucleus in red cell function of the many species with nucleated erythrocytes has never been determined.

Summary

Measurements of erythrocytes and their nuclei were made in 8 species of fishes, 5 species of Amphibia, and 33 species of reptiles. The cytosomes of elasmobranchs are almost twice the size of those in teleosts, being 19 μ to 20 μ by 12 μ to 14 μ. The cytosomes of *Opsanus* and *Lophius* are almost as wide as long, while in other fishes they are much longer than wide. The cytosome measurements in the two anurans, *Hyla* and *Bufo*, are similar to those in elasmobranchs while those in *Rana* are larger. Cytosomes in the crocodile, turtles and some lizards are also similar in size to those in the elasmobranchs. The cytosomes of snakes are large (15.5 μ to 23 μ), those of *Bothrops* being the largest of all reptiles studied. Based on the assumption that the least diameter of the erythrocyte indicated the minimal diameter of the capillaries, the largest capillaries are found in the elasmobranchs and the smallest in the teleosts.

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